(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 October 2005 (20.10.2005)

PCT

(10) International Publication Number
WO 2005/098046 A2

(51) International Patent Classification[7]: C12Q 1/68

(21) International Application Number:
PCT/US2005/010940

(22) International Filing Date: 31 March 2005 (31.03.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/558,520 1 April 2004 (01.04.2004) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2005/098046 A2

(54) Title: METHODS FOR THE DETERMINATION OF CELL SPECIFIC BIOMARKERS

(57) Abstract: The present invention provides methods for the reduction of the considerate amount of white cell background that interferes with meaningful analysis of a patient's food sample when the analysis involves rare cell analysis. Nucleic acid profile analysis of targeted rare cells is obtained from an individual patient's enriched blood sample by subtracting the white cell nucleic acid content from the same enriched sample, prior to positive selection of the target cell content. Subsequent profile analysis of the remaining nucleic acids allow for specific mRNA expression profiles having improved signal-to-noise. The methods are useful in profiling of cells isolated from tissues or body fluids and serves as an adjunct to clinical diagnosis of diverse carcinomas including early stage detection and classification of circulating tumor cells. Monitoring of nucleic acid and protein profiles of cells either in conventional or microarray formats, facilitates management of therapeutic intervention including staging, monitoring response to therapy, confirmation of remission and detection of regression.

Title: Methods for the Determination of Cell Specific Biomarkers.

Inventors: Shawn Mark O'Hara and Denis Smirnov

Background of the Invention

Field of the Invention

This invention relates generally to gene specific amplification, analysis and profiling of cytosolic biomolecules useful in the fields of oncology, diagnostic testing and pharmacogenomics (personalized medicine). The invention is particularly useful in such fields as cancer screening, selecting (identification and stratification of therapy responders / non-responders) and monitoring for chemotherapy treatment, or cancer recurrence. More specifically, the present invention facilitates comprehensive analysis of mRNA and DNA from rare target cells. To accomplish this, the invention acts to subtract the white blood cell (WBC) noise from genetic markers associated with target rare cells.

Description of Related Art

Any given cell will express only a fraction of the total number of genes present in its genome. A portion of the total number of genes that are expressed determine aspects of cell function such as development and differentiation, homeostasis, cell cycle regulation, aging, apoptosis, etc. Alterations in gene expression decide the course of normal cell development and the appearance of disease states, such as cancer. The expression of specific genes will have a profound effect on the nature of any given cell and its response to specific therapies. Accordingly, the methods of analyzing gene expression, as such as those provided by the present invention are important in basic molecular biological research and improved disease management for individuals. Identification of specific genes, especially rare genes, can provide a key to diagnosis, prognosis and treatment for a variety of diseases that reflect these expression levels (Levsky, et al., Single-Cell Gene Expression Profiling, Science, 297:836-840, (2002)).

Differential gene expression is a commonly used method of assessing gene expression in a cell. In particular, cDNA microarray analysis compares cDNA target sequence levels obtained from cells or organs from healthy and diseased individuals. These targets are then hybridized to a set of probe fragments immobilized on a membrane. Differences in the resultant hybridization pattern are then detected and related to differences in gene expression of the two sources (US 6,383,749). Competing events such as interactions between non-complementary target sequences nonspecific binding between target and probe and secondary structures in target sequences will interfere with hybridization and result in a decline of the signal-to-noise.

While gene specific primer sets have been used to selectively amplify a specific subset of mRNA from an mRNA library, there exists a clear need to reduce the signal-to-noise ratio in an amplification process which is especially applicable in rare cell detection for diagnostic therapy to encompass both quantitative and qualitative analysis.

Rare cells, such as circulating tumor cells (CTC), represent a surrogate source of tissue in the diagnosis, prognosis and treatment of disease (US 6,645,731; US 6,365,362; 10/079,939; 10/269,579). Further, advancements in the detection, phenotyping and genotyping will expand the clinical utility of such cells and may lead to therapies tailored to individual patients. It is generally accepted that the presence of circulating tumor cells (CTC) in a patient's blood provides an early detection system in assessing the need for therapeutic intervention. Highly sensitive assays to allow accurate enumeration of circulating carcinoma cells have shown that the peripheral blood tumor cell load correlate with disease state (Terstappen et al., Peripheral Blood Tumor Cell Load Reflects the Clinical Activity of the Disease in Patients with Carcinoma of the Breast, International J. of Oncology., 17:573-578, 2000).

Charting gene expression patterns of rare cell events (e.g. CTC) through microarray analysis of gene expression levels would be a desirable indicator of tumor properties in other diseases such as lymphomas, acute leukemia,

breast cancer, prostate cancer, lung cancer and liver cancer etc. However, to discover and adapt this genetic information for patient management use requires resolution of inherent significant signal-to-noise issues in present state-of-the-art technology.

One of the most pressing goals of rare cell detection research is to identify a set of markers that allow efficient detection and prognosis of these cells in the peripheral blood of patients, having these cells. In addition to simply detecting the presence in peripheral blood, some markers can also provide useful information about the tissue of origin and potentially serve as a predictor of clinical outcome for a patient and a selection guide for the most efficient therapeutic agent. Continuing detection and characterization can help to track a treatment progress of the cancer patients. The expression of the marker gene as minimal or absent in the blood cells other than the target rare cells provides for a clear signal.

A reliable method of standardized WBC subtraction of nucleic acid noise from the target genetic markers provides an unmeet need in the analysis of gene expression. This is especially true where fast hybridization, highly specific binding of targets to complementary probes, and substantially improved signal-to-noise ratios are used in rare cell detection and analysis. Consequently, the present invention has additional importance when assessing gene expression as it relates to cancer and disease related states as well as in rare circulating endothelial cell (CEC) events associated with cardiovascular disease (see US App. 10/079,939 and US App. 09/904,472 both of which are fully incorporated by reference herein).

Summary of the Invention

The present invention provides methods for detecting genetic information of rare cells in a biological sample, which methods generally comprise:

a. obtaining a biological sample containing a mixed population of cells from an individual suspected of having target rare cells;

b. fractionating said biological sample to obtain a fraction suspected of containing said rare cells;

c. assessing said fraction for a first gene profile;

d. separating said rare cells from said fraction whereby a depleted fraction is devoid of said rare cells;

e. determining a second gene profile of said depleted fraction; and

f. subtracting said second gene profile from said first gene profile to obtain said genetic information from said rare cells.

In a preferred embodiment of the invention, the method involves selecting the rare cells from a group consisting of cancer cells, epithelial cells, endothelial cells, activated T-lymphocyte cells, dendritic cells and combinations thereof.

The present invention also provides methods for the reduction of the considerable amount of white cell background that interferes with meaningful analysis of a patient's blood sample when the analysis involves rare cell analysis. Nucleic acid profile analysis of targeted rare cells is obtained from an individual patient's enriched blood sample by subtracting the white cell nucleic acid content from the same enriched sample, prior to positive selection of the target cell content. Subsequent profile analysis of the remaining nucleic acids allow for specific mRNA expression profiles having improved signal-to-noise.

The methods of the invention are useful in profiling of cells isolated from tissues or body fluids and serves as an adjunct to clinical diagnosis of diverse carcinomas including early stage detection and classification of circulating tumor cells. Monitoring of nucleic acid and protein profiles of cells either in conventional or microarray formats, facilitates management of therapeutic intervention including staging, monitoring response to therapy, confirmation of remission and detection of regression.

Brief Description of the Drawings

Figure 1 illustrates the mRNA expression levels of CK19, PSA, PSM, AR, Hepsin, HK2, PSGR, MGB1 and MGB2 in the mRNA libraries from 23 samples of CTC enriched from 9 metastatic cancer patients.

Figure 2 illustrates the mRNA expression levels of 37 genes listed and demonstrated the fundamental problem with current mRNA analysis of Ficoll/Percoll or immunomagnetically enriched CTC/CEC in that WBC or nonspecific binding of WBC confounds and limits the breadth and depth of genes that can be measured in a meaningful manor. As can be see only 12 of the 37 genes of interest were able to be measured with out any interference from the donor WBC population (AR, CEA, CK5, CK19, EGFR, ER-b, HK2, MGB1, MGB2, PSA, PSGR, PSM, TROP2). As a result of this only a sub set there of 9 genes were then applied to advanced prostate cancer as shown in Figure 1. The other 24 genes could not be measures (NKX3A-CK10) due levels of these genes expressed in WBC nonspecifically bound to the epithelial specific immunomagnetic beads.

Detailed Description of the Invention

Because of the considerable amount of white cell background in mRNA profile analysis of rare cells, methods are presented to provide meaningful analysis of patient blood samples containing rare circulating target cells such as CTC and/or CEC. These methods provide an individual patient-matched comparison between the combined genetic information (CTC, CEC and blood sample WBC) and genetic information after CTC and/or CEC depletion (WBC blood sample only). For example in assessing the genetic profile of those patients diagnosed with a particular cancer and suspected of having circulating tumor or endothelial cells, eliminating the WBC background in a sample provides a meaningful reduction in the noise component enabling substantially improved analysis of genetic signals from the rare cells. Currently known methods are limited to amplification of individual genes that provide the least background noise, and do not consider other genes that have substantial background interference yet may be relevant to a particular disease material. Thus by subtracting an individual patient's WBC gene profile from the same individual patient's CTC and/or CEC gene profile, a meaningful detection system is described to allow assessment of relevant specific genetic information. This can be performed on a single gene basis with, multiplex gene analysis or global analysis of transcriptome/proteome/genome such as with massively parallel probe arrays.

Using the method of the present invention, expression profiling of genetic information is improved with the subtraction of background genetic information obtained from the same individual patient's WBC. This genetic profile is subtracted from the same blood sample, leaving only the genetic information from the target cells to further analyze. More specifically, an enriched fraction of whole blood is immunomagnetically enriched as previously described (US 6,365,362; US 6,645,731; US 10/079,939; US 10/269,579). The target cells are positively selected using antibodies specific to target cell antigens which are most often surface antigens. The remaining fraction, containing the depleted target cells, is assayed separately and compared to the same

enriched patient blood sample fraction, prior to positive selection by array analysis or by RT-PCR etc.

Further the subtraction of WBC noise (e.g. nonspecifically enriched cells carried over due to process) provides a unique target cell specific panel of genes. These genes are consistently found in disease groups suggesting an important role in the diagnosis and management of diseases linked to the circulating rare cells. More specifically, diseases such as colorectal cancer, breast cancer prostate cancer and any combinations thereof can be screened for unique after early detection.

As used herein, the following terms are defined as follows:

"Cytoplasmic biomolecules" includes cellular target molecules of interest such as, but not limited to, protein, polypeptides, glycoprotein, oligosaccharide, lipids, electrolytes, RNA, DNA and the like, that is located in the cytoplasmic compartment of a cell. Upon contacting a cell with a permeabilization compound and subsequent cell separation, the cytoplasmic biomolecules are present in the supernatant for downstream analysis. All soluble cytoplasmic biomolecules, for example, the entire cytoplasmic RNA library or target components capable of traversing the membrane pores can be isolated and analyzed. In a preferred embodiment, the focus is on the analysis of transcribed mRNA and translated proteins, for example in CTC, as indicators of oncogenic transformations of interest in the management of cancer diagnosis and therapy.

"Membrane biomolecules" includes any extracellular, intra-membrane, or intracellular domain molecule of interest that is associated with or imbedded in the cell membranes including, but not limited to, the outer cell membrane, nuclear membrane, mitochondrial and other cellular organelle membranes. Upon permeabilization with a permeabilization compound of this invention, the targeted membrane biomolecules are normally not solubilized or removed from the membrane, i.e. the membrane biomolecules remain associated with the permeabilized cell. Membrane biomolecules include, but are not limited to, proteins, glycoproteins, lipids, carbohydrates, nucleic acids and

combinations thereof, that are associated with the cellular membrane, including those exposed on the external or extracellular surface of the outer membrane as well as those that are present on the internal surface of the outer membrane, and those proteins associated with the nuclear, mitochondrial and all other intracellular organelle membranes. Membrane biomolecules also include cytoskeletal proteins.

Morphology in reference to cell structure is used as customarily defined, pertaining to cell and nuclear topology and surface characteristics including intracellular or surface markers or epitopes permitting staining with histochemical reagents or interaction with detectably labeled binding partners such as antibodies. In addition morphology shall include the entire field of "morphometry" defined as: quantitative measure of chromatin distribution within the nucleus.

The terms genomic and proteomic are used as conventionally defined. "Functional" is herein used as an adjective for an empirically detectable biological characteristic or property of a cell such as "functional cellomic" which more broadly encompasses both genomic and proteomic as well as other target categories including, but not limited to, "glyconomic" for carbohydrates and "lipidomic" for cellular lipids. The resultant cell characteristics provide profiles permitting differentiation of normal from transformed cells.

"Contacting" means bringing together, either directly or indirectly, a compound or reagent into physical proximity of a cell. The cell and/or compounds can be present in any number of buffers, salts, solutions, etc. Contacting includes, for example, placing the reagent solution into a tube, microtiter plate, microarray, cell culture flask, or the like, for containing the cell(s). The microtiter plate and microarray formats further permit multiplexed assays for simultaneously analyzing a multiplicity of cellular target compounds or components including, but not limited to, nucleic acids and proteins.

"Permeabilization compound, reagent, or composition" means any reagent that forms small pores in the cell membranes, comprising the lipid-cholesterol bilayer, while maintaining sufficient membrane, cytoplasmic and nuclear

structure such that subsequent phenotypic analysis can be carried out on the permeabilized cell(s). For example, saponin is a known "pore-forming" compound that complexes with cell membrane components thereby forming numerous trans-membrane pores of about 8 nm size in the cell wall or membrane, thus allowing outward diffusion of small soluble cytosolic constituents, such as enzymes, proteins, glycoproteins, globulins, electrolytes, and the like, and internal equilibration with extracellular reagent components, such as electrolytes.

"Immunomagnetic beads" are magnetically labeled nanoparticles or microparticles also having covalently attached binding reagents (e.g. antibodies) with substantially selective affinity for surface markers or epitopes on cells, thereby achieving selective capture of magnetically labeled cells when exposed to a magnetic field such as generated in high gradient magnetic separation system (HGMS). Other terms used herein for methodologies, reagents and instruments are as conventionally defined and known to persons skilled in the art.

Description of Preferred Embodiments

As has been indicated in the foregoing discussion, a more comprehensive and practical form of cancer diagnosis must also include analysis of intra- and extra-cellular membrane antigens as well as analysis of cellular RNA content and DNA content in the same cell or cell population (US 6,365,362).

One of the many applications of this type of cell analysis is in cancer diagnostics. Many clinicians believe that cancer is an organ specific disease when confined to its early stages. The disease becomes systemic by the time it is first detected using methods currently available. Accordingly, evidence to suggest the presence of tumor cells in the circulation would provide a first line detection mechanism that could either replace, or function in conjunction with other tests such as mammography or measurements of prostate specific antigen. By analyzing cellular phenotype (protein and RNA) and genotype through specific markers for these cells, the organ origin of such cells may readily be determined, e.g., breast, prostate, colon, lung, ovarian or other non-hematopoietic cancers. Thus in situations where protein RNA and genome

can be analyzed, especially where no clinical signs of a tumor are available, it is possible to identify the presence of a specific tumor as well as the organ of origin. As these profiles define cell function, they also indicate what the most appropriate therapy type and course should be when used in cancer cell detection. Further in monitoring cases where there is no detectable evidence of circulating tumor cells as with post operative surgery or other successful therapies, it may be possible to determine from a further clinical study whether further treatment is necessary.

Generally, the profiling of any targeted rare event after subtraction of an enriched sample is considered in this invention. Accordingly, hormones, proteins, peptides, lectins, oligonucleotides, drugs, chemical substances, nucleic acid molecules (such as RNA and/or DNA), bioparticles such as cells, apoptotic bodies, cell debris, nuclei, mitochondria, viruses, bacteria, and the like would be included in the embodiment of this invention. Enrichment of the target event can be accomplished by any means known in the art, but preferably immunomagnetic enrichment. After subtraction of the combined cytoplasmic biomolecule population in the enriched sample from the biomolecule population in the rare event, a profile analysis of the remaining signals is used as a descriptive index of the rare event.

The fluid sample includes, without limitation, cell-containing bodily fluids, peripheral blood, bone marrow, urine, saliva, sputum, semen, tissue homogenates, nipple aspirates, and any other source of rare cells that is obtainable from a human subject.

One method of providing for a more comprehensive diagnosis, embodied in the present invention, is the profiling of nucleic acids uniquely identified in circulating rare cells that are found in whole blood in a rapid, dependable, and standardized procedure. To this end, a whole blood sample is obtained to magnetically enrich the cytoplasmic biomolecules from a cell or population of cells from an individual patient to yield a fraction containing WBC and rare cells. The rare cells are positively selected, and the remaining enriched fraction is assayed on an array. This array is subtracted from the initially enriched sample to yield a genetic profile of the rare cell.

Gene expression targets (mRNA) for identifying tissue of origin, diagnosis, prognosis, therapy target characterization and monitoring include but are not limited to cells derived from cancers of the breast, prostate, lung, colon, ovary, kidney, bladder, and the like for the purpose of detection and monitoring of sensitive or resistant genes expressing markers such as mammoglobin 1 (MGB1), mammoglobin 2 (MGB2), prolactin inducible protein (PIP), carcinoembryonic antigen (CEA), prostate specific antigen (PSA), prostate specific membrane antigen (PSMA), glandular kallikrein 2 (hK2), androgen receptor (AR), prostasin, Hespin (HPN), DD3, Her-2/Neu, BCL2, epidermal growth factor receptor (EGFR), tyrosine kinase-type receptor (HER2), thymidylate synthetase (TS), vascular endothelial growth factor VEGF, pancreatic mucin (Muc1), guanylyl cyclase c (GC-C), phosphatidylinositol 3 kinase (PIK3CG), protein kinase B gamma (AKT), excision repair protein (ERCC1), alpha-1 globin (F6), macrophage inhibitory cytokin-1 (G6), dihydropyrimidine dehydrogenase (DPYD), insulin growth factor receptor (IGF2) estrogen receptors alpha and beta (ER), progesterone receptor (PR), aromatase (cyp19), Telomerase (TERT), general epithelial tissue specific genes, cytokeratin 19 (CK19), cytokeratin 5 (CK5), cytokeratin 8 (CK8), cytokeratin 10 (CK10), cytokeratin 20 (CK20), epithelial cell adhesion molecule (EpCAM), mucins including mucin 1 (MUC1), topoisomerases, urokinase plasminogen activator (uPA), urokinase plasminogen activator receptor (uPAR), matrix metalloproteinases (MMP), general white blood cell specific mRNA, alpha-1-globin, CD16, CD45, and CD31, and the like. This list is intended to illustrate the general diversity of arrays of mRNA-specific genes that could be assembled to differentiate cells from diverse origins, types and diseases, and is not intended to be comprehensive.

From a previously disclosed invention commonly assigned herewith, US Patent No. 6,365,362 and US App. Serial No.10/079,939 (both of which are incorporated by reference herein), circulating epithelial cells can be enriched relative to leukocytes to the extent of at least 2,500 fold to around 10,000 fold. Immunomagnetic selection of circulating epithelial cells in blood is followed by nucleotide analysis embodied in this invention. The enrichment is only one

example of many methods known in the art for selecting specific populations of cells to be used in the embodiment of this invention.

Immunomagnetic enrichment of circulating tumor cells provides a 4 to 5 log unit reduction in leukocytes, the typical range of CTC to leukocytes is 1-10 CTC per 10^4 leukocytes. The low number of CTC's and the leukocyte carried over during the CTC enrichment process pose significant detection restrictions in the signal-to-noise, constraining the choice of genes and gene expression profiling methods (Figure 2). Therefore, subtraction of the leukocytes from the sample would minimize the affect on the signal-to-noise.

Positive selection of cytoplasmic biomolecules such as target rare cells is accomplished through immunomagnetic selection with an antibody specific for the target cell. The nucleic acid content of the remaining sample is profiled and subtracted from the profile of the initial sample, prior to positive selection.

Profile analysis of cytoplasmic RNA (and other RNA such as mtRNA and hnRNA), DNA, and protein based analysis techniques. These include all types of cDNA, RNA and protein microarrays for profile analyses, mass spectrometry, fluorescent *in situ* hybridization (FISH), single nucleotide polymorphism (SNP), all genomic-based amplification techniques such as PCR and the like, microsatellite analysis, restriction fragment length polymorphism (RFLP, ALFP), SAGE, DD-RT-PCR, and the like.

Such analyses can be conducted on as few as 1-10 RNA molecules for each and any RNA sequence type, but preferably on tens of thousands up to millions copies of targets to enable detection of subtle alterations in cellular translation or transcription profiles as indicators of disease states in a clinical setting. Other functional cell profiles of releasable and non-releasable cellular components, such as proteins, glycoproteins, lipoproteins, oligoglycosides and the like, can similarly be generated by analyzing the two fractions by conventional microarray, HPLC, electrophoretic methods including the high-resolution 2D electrophoresis method, or antibody array profiling.

The following examples are provided to exemplify the practicality of the disclosed invention and to demonstrate the impact of the invention on

diagnostic technology. These examples are not intended to limit the scope of the invention. In addition, the disclosures of each patent, patent application, and publication cited or described in this document are incorporated herein by reference in the entirety.

EXAMPLE 1

mRNA EXPRESSION OF MULTIPLE GENES IN CTCs

The characterization of CTC is further improved over cell enumeration as it is feasible to profile nucleic acid content in these cells by in vitro transcription based RT-PCR expression obtained from patients with hormone refractory prostate cancer (HRPC). Expression of 37 genes with potential utility for epithelial cell characterization was evaluated from antisense RNA (aRNA) libraries constructed from immunomagnetically enriched CTC from 7.5 ml of blood samples from healthy donors and HRPC.

The results showed no expression in 13 of 37 genes in the control group. Of the genes expressed in the CTC from the 23 blood specimens drawn from 9 metastatic prostate cancer patients were CK 19 18/23 (78%), PSA 20/23 (89%), PSM 17/23 (74%), AR 16/23 (70%), hK2 7/23 (30%), EGFR 4/23 (17%), and PSGR 2/23 (9%). The number of CTC in these samples ranged from 4 to 283 per 7.5 mL blood (mean 87, median 89). Some of the genes had a low level of expression in the control samples and were expressed at higher levels in the patient samples. In all 23 samples CK19, EpCAM or Muc-1 was expressed. Due to background expression in the controls, expression of 13 of the 37 genes including HER-2, p53 and BCL-2 could not be measured in CTCs (Figure 1)

From these results, aRNA libraries can be constructed from CTCs and gene expression profiles of CTCs were obtained in HRPC. This can enhance characterization of HRPC and facilitate the development of more effective therapies in HRPC.

EXAMPLE 2

Assessment of Microarray Chip Selectivity

Several clinical sample types and a model cell line system were assessed. Affymetrix Focus 8,700 gene microarray chips were evaluated using two test systems. One system is composed of actual patient samples where CTC and WBC were predetermined by Flow. The other test system is a reconstituted cell line model system (LN-CAP/ZR75 mixture) having known copy numbers of nine different CTC mRNA species.

With the cell line model system, gene expression was detected down to range of about 140-800 copies of specific mRNA present, following immunomagnetic enrichment. This sensitivity result approximately equals the Affymetrix claimed sensitivity of $1/10^5$. Thus if 10 or more CTC are present in a sample, the sensitivity translates into an ability to detect a substantial number of gene sequences when present at about 50-100 copies per cell (i.e. 50-100 copies/cell x 10 CTC = 500-1000 copy signal) and suggests the successful application to rare cell events in blood such as circulating tumor or endothelial cell mRNA profiling.

The second test system utilized clinical containing samples from patients with known cancers. Hybridization with samples from patients with advanced prostate cancer (650 CTC's) and colon cancer (105 CTC's) revealed a set of genes that are upregulated in CTC samples, after subtraction of the depleted background.

EXAMPLE 3

Microarray Expression Analysis in Genes with No Detectable Expression Following Depletion of CTC's

Analysis of genes detected prior to depletion of the WBC fraction and not after subtraction of the WBC genetic information resulted in sets of genes identified only through their expression in CTC's exclusively from breast, prostate, or colorectal cancers. Gene sets were also identified in exclusive combinations of cancer patients (i.e. breast and colorectal, breast and prostate, prostate and colorectal), and in general expressed in all three cancers.

Affymetrix Focus 8,700 gene microarray chips were used after individual patient WBC subtraction by immunomagnetic selection. Table 1 shows 322 genes identified from individual patients diagnosed with cancer. Each Affymetrix chip contains over 8000 full-length human transcripts that are commercially available for screening. Patients diagnosed with breast cancer showed 86 positives unique for breast cancer. Patients with diagnosed prostate cancer had 60 positives unique for prostate cancer, and patients with colorectal cancer had 74 positives unique for colorectal cancer. Further, 32 genes were positive for both breast and prostate cancers, 17 genes were positive for breast and colorectal cancer, 10 genes were positive for prostate and colorectal cancer, and 43 genes were positive for breast prostate and colorectal.

Table 1: Genetic profile of genes not detected in the depleted WBC portion.

Affymetrix ID	Gene Name	Gene Symbol	Predicted Gene Expression CTC Specificity
205979_at	secretoglobin, family 2A, member 1	SCGB2A1	Breast cancer
202575_at	cellular retinoic acid binding protein 2	CRABP2	Breast cancer
209016_s_at	keratin 7	KRT7	Breast cancer
205916_at	S100 calcium binding protein A7 (psoriasin 1)	S100A7	Breast cancer
206799_at	secretoglobin, family 1D, member 2	SCGB1D2	Breast cancer
205980_s_at	Rho GTPase activating protein 8	ARHGAP8	Breast cancer
204734_at	keratin 15	KRT15	Breast cancer
214451_at	transcription factor AP-2 beta (activating enhancer binding protein 2 beta)	TFAP2B	Breast cancer
204818_at	hydroxysteroid (17-beta) dehydrogenase 2	HSD17B2	Breast cancer
204041_at	monoamine oxidase B	MAOB	Breast cancer
204400_at	embryonal Fyn-associated substrate	EFS	Breast cancer
203963_at	carbonic anhydrase XII	CA12	Breast cancer
221024_s_at	solute carrier family 2 (facilitated glucose transporter), member 10	SLC2A10	Breast cancer
201015_s_at	junction plakoglobin	JUP	Breast cancer
206509_at	prolactin-induced protein	PIP	Breast cancer
41660_at	cadherin, EGF LAG seven-pass G-type receptor 1 (flamingo homolog, Drosophila)	CELSR1	Breast cancer
220414_at	calmodulin-like skin protein	CLSP	Breast cancer
205319_at	prostate stem cell antigen	PSCA	Breast cancer
221872_at	retinoic acid receptor responder (tazarotene induced) 1	RARRES1	Breast cancer
202357_s_at	B-factor, properdin	BF	Breast cancer
204379_s_at	fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism)	FGFR3	Breast cancer
218976_at	J domain containing protein 1	JDP1	Breast cancer
208165_s_at	protease, serine, 16 (thymus)	PRSS16	Breast cancer
36499_at	cadherin, EGF LAG seven-pass G-type receptor 2 (flamingo homolog, Drosophila)	CELSR2	Breast cancer
204284_at	protein phosphatase 1, regulatory (inhibitor) subunit 3C	PPP1R3C	Breast cancer
204679_at	Potassium channel, subfamily K, member 1	KCNK1	Breast cancer
203029_s_at	protein tyrosine phosphatase, receptor type,	PTPRN2	Breast cancer

	N polypeptide 2		
220625_s_at	E74-like factor 5 (ets domain transcription factor)	ELF5	Breast cancer
202871_at	TNF receptor-associated factor 4	TRAF4	Breast cancer
823_at	chemokine (C-X3-C motif) ligand 1	CX3CL1	Breast cancer
218587_s_at	x 010 protein	MDS010	Breast cancer
216836_s_at	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	ERBB2	Breast cancer
203815_at	glutathione S-transferase theta 1	GSTT1	Breast cancer
205066_s_at	ectonucleotide pyrophosphatase/phosphodiesterase 1	ENPP1	Breast cancer
205242_at	chemokine (C-X-C motif) ligand 13 (B-cell chemoattractant)	CXCL13	Breast cancer
207417_s_at	zinc finger protein 177	ZNF177	Breast cancer
219793_at	sorting nexin 16	SNX16	Breast cancer
204306_s_at	CD151 antigen	CD151	Breast cancer
210096_at	cytochrome P450, family 4, subfamily B, polypeptide 1	CYP4B1	Breast cancer
218967_s_at	phosphotriesterase related	PTER	Breast cancer
205286_at	transcription factor AP-2 gamma (activating enhancer binding protein 2 gamma)	TFAP2C	Breast cancer
212724_at	ras homolog gene family, member E	ARHE	Breast cancer
206793_at	phenylethanolamine N-methyltransferase	PNMT	Breast cancer
204942_s_at	aldehyde dehydrogenase 3 family, member B2	ALDH3B2	Breast cancer
205225_at	estrogen receptor 1	ESR1	Breast cancer
211421_s_at	ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease)	RET	Breast cancer
201292_at	topoisomerase (DNA) II alpha 170kDa	TOP2A	Breast cancer
205266_at	leukemia inhibitory factor (cholinergic differentiation factor)	LIF	Breast cancer
204497_at	adenylate cyclase 9	ADCY9	Breast cancer
206714_at	arachidonate 15-lipoxygenase, second type	ALOX15B	Breast cancer
202894_at	EphB4	EPHB4	Breast cancer
219274_at	transmembrane 4 superfamily member tetraspan NET-2	NET-2	Breast cancer
206539_s_at	cytochrome P450, family 4, subfamily F, polypeptide 12	CYP4F12	Breast cancer
202431_s_at	v-myc myelocytomatosis viral oncogene	MYC	Breast cancer

	homolog (avian)		
204078_at	nucleolar autoantigen (55kD) similar to rat synaptonemal complex protein	SC65	Breast cancer
204032_at	breast cancer anti-estrogen resistance 3	BCAR3	Breast cancer
202743_at	phosphoinositide-3-kinase, regulatory subunit, polypeptide 3 (p55, gamma)	PIK3R3	Breast cancer
203002_at	angiomotin like 2	AMOTL2	Breast cancer
207056_s_at	solute carrier family 4, sodium bicarbonate cotransporter, member 8	SLC4A8	Breast cancer
205258_at	inhibin, beta B (activin AB beta polypeptide)	INHBB	Breast cancer
208626_s_at	vesicle amine transport protein 1 homolog (T californica)	VAT1	Breast cancer
205453_at	homeo box B2	HOXB2	Breast cancer
218665_at	frizzled homolog 4 (Drosophila)	FZD4	Breast cancer
203929_s_at	microtubule-associated protein tau	MAPT	Breast cancer
57540_at	ribokinase	RBSK	Breast cancer
214600_at	TEA domain family member 1 (SV40 transcriptional enhancer factor)	TEAD1	Breast cancer
209610_s_at	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	SLC1A4	Breast cancer
204453_at	zinc finger protein 84 (HPF2)	ZNF84	Breast cancer
35148_at	tight junction protein 3 (zona occludens 3)	TJP3	Breast cancer
205181_at	zinc finger protein 193	ZNF193	Breast cancer
205352_at	serine (or cysteine) proteinase inhibitor, clade I (neuroserpin), member 1	SERPINI1	Breast cancer
205809_s_at	Wiskott-Aldrich syndrome-like	WASL	Breast cancer
202338_at	thymidine kinase 1, soluble	TK1	Breast cancer
221584_s_at	potassium large conductance calcium-activated channel, subfamily M, alpha member 1	KCNMA1	Breast cancer
218311_at	mitogen-activated protein kinase kinase kinase kinase 3	MAP4K3	Breast cancer
217974_at	seven transmembrane protein TM7SF3	TM7SF3	Breast cancer
213110_s_at	collagen, type IV, alpha 5 (Alport syndrome)	COL4A5	Breast cancer
213030_s_at	plexin A2	PLXNA2	Breast cancer
205759_s_at	sulfotransferase family, cytosolic, 2B, member 1	SULT2B1	Breast cancer
206346_at	prolactin receptor	PRLR	Breast cancer
204378_at	breast carcinoma amplified sequence 1	BCAS1	Breast cancer

203426_s_at	insulin-like growth factor binding protein 5	IGFBP5	Breast cancer
205355_at	acyl-Coenzyme A dehydrogenase, short/branched chain	ACADSB	Breast cancer
205190_at	plastin 1 (I isoform)	PLS1	Breast cancer
204783_at	myeloid leukemia factor 1	MLF1	Breast cancer
208078_s_at	transcription factor 8 (represses interleukin 2 expression)	TCF8	Breast cancer
209854_s_at	kallikrein 2, prostatic	KLK2	Prostate Cancer
217771_at	golgi phosphoprotein 2	GOLPH2	Prostate Cancer
210297_s_at	microseminoprotein, beta-	MSMB	Prostate Cancer
207030_s_at	cysteine and glycine-rich protein 2	CSRP2	Prostate Cancer
206001_at	neuropeptide Y	NPY	Prostate Cancer
205924_at	RAB3B, member RAS oncogene family	RAB3B	Prostate Cancer
203180_at	aldehyde dehydrogenase 1 family, member A3	ALDH1A3	Prostate Cancer
202363_at	sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican)	SPOCK	Prostate Cancer
205311_at	dopa decarboxylase (aromatic L-amino acid decarboxylase)	DDC	Prostate Cancer
210576_at	cytochrome P450, family 4, subfamily F, polypeptide 8	CYP4F8	Prostate Cancer
204934_s_at	hepsin (transmembrane protease, serine 1)	HPN	Prostate Cancer
201110_s_at	thrombospondin 1	THBS1	Prostate Cancer
206167_s_at	Rho GTPase activating protein 6	ARHGAP6	Prostate Cancer
213793_s_at	homer homolog 1 (Drosophila)	HOMER1	Prostate Cancer
205968_at	potassium voltage-gated channel, delayed-rectifier, subfamily S, member 3	KCNS3	Prostate Cancer
210163_at	chemokine (C-X-C motif) ligand 11	CXCL11	Prostate Cancer
214596_at	ESTs		Prostate Cancer
211110_s_at	androgen receptor (dihydrotestosterone receptor; testicular feminization; spinal and bulbar muscular atrophy; Kennedy disease)	AR	Prostate Cancer
205051_s_at	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	KIT	Prostate Cancer
220116_at	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2	KCNN2	Prostate Cancer
207469_s_at	Pirin	PIR	Prostate Cancer
33767_at	neurofilament, heavy polypeptide 200kDa	NEFH	Prostate Cancer
202237_at	nicotinamide N-methyltransferase	NNMT	Prostate Cancer

205509_at	carboxypeptidase B1 (tissue)	CPB1	Prostate Cancer
201976_s_at	myosin X	MYO10	Prostate Cancer
202133_at	transcriptional co-activator with PDZ-binding motif (TAZ)	TAZ	Prostate Cancer
203557_s_at	6-pyruvoyl-tetrahydropterin synthase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1)	PCBD	Prostate Cancer
205548_s_at	BTG family, member 3	BTG3	Prostate Cancer
212589_at	related RAS viral (r-ras) oncogene homolog 2	RRAS2	Prostate Cancer
220474_at	solute carrier family 25 (mitochondrial oxodicarboxylate carrier), member 21	SLC25A21	Prostate Cancer
208546_x_at	histone 1, H2bh	HIST1H2BH	Prostate Cancer
220004_at	DEAD-box protein	HAGE	Prostate Cancer
201614_s_at	RuvB-like 1 (E. coli)	RUVBL1	Prostate Cancer
201117_s_at	carboxypeptidase E	CPE	Prostate Cancer
212224_at	aldehyde dehydrogenase 1 family, member A1	ALDH1A1	Prostate Cancer
204321_at	neogenin homolog 1 (chicken)	NEO1	Prostate Cancer
200771_at	laminin, gamma 1 (formerly LAMB2)	LAMC1	Prostate Cancer
203356_at	Homo sapiens cDNA FLJ36423 fis, clone THYMU2011308.		Prostate Cancer
202779_s_at	ubiquitin carrier protein	E2-EPF	Prostate Cancer
202996_at	polymerase (DNA-directed), delta 4	POLD4	Prostate Cancer
206610_s_at	coagulation factor XI (plasma thromboplastin antecedent)	F11	Prostate Cancer
206239_s_at	serine protease inhibitor, Kazal type 1	SPINK1	Prostate Cancer
205680_at	matrix metalloproteinase 10 (stromelysin 2)	MMP10	Prostate Cancer
210720_s_at	amyloid beta (A4) precursor protein-binding, family A, member 2 binding protein	APBA2BP	Prostate Cancer
219954_s_at	glucosidase, beta, acid 3 (cytosolic)	GBA3	Prostate Cancer
216958_s_at	isovaleryl Coenzyme A dehydrogenase	IVD	Prostate Cancer
34478_at	RAB11B, member RAS oncogene family	RAB11B	Prostate Cancer
209291_at	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	ID4	Prostate Cancer
210502_s_at	peptidylprolyl isomerase E (cyclophilin E)	PPIE	Prostate Cancer
209621_s_at	alpha-actinin-2-associated LIM protein	ALP	Prostate Cancer
208453_s_at	X-prolyl aminopeptidase (aminopeptidase P) 1, soluble	XPNPEP1	Prostate Cancer
207065_at	cytokeratin type II	K6HF	Prostate Cancer

221437_s_at	mitochondrial ribosomal protein S15	MRPS15	Prostate Cancer
205833_s_at	Prostate androgen-regulated transcript 1	PART1	Prostate Cancer
202980_s_at	seven in absentia homolog 1 (Drosophila)	SIAH1	Prostate Cancer
205463_s_at	platelet-derived growth factor alpha polypeptide	PDGFA	Prostate Cancer
205901_at	prepronociceptin	PNOC	Prostate Cancer
203717_at	dipeptidylpeptidase 4 (CD26, adenosine deaminase complexing protein 2)	DPP4	Prostate Cancer
211558_s_at	deoxyhypusine synthase	DHPS	Prostate Cancer
202799_at	ClpP caseinolytic protease, ATP-dependent, proteolytic subunit homolog (E. coli)	CLPP	Prostate Cancer
205819_at	macrophage receptor with collagenous structure	MARCO	Colorectal Cancer
210220_at	frizzled homolog 2 (Drosophila)	FZD2	Colorectal Cancer
207850_at	chemokine (C-X-C motif) ligand 3	CXCL3	Colorectal Cancer
205892_s_at	Fatty acid binding protein 1, liver	FABP1	Colorectal Cancer
202949_s_at	four and a half LIM domains 2	FHL2	Colorectal Cancer
204259_at	matrix metalloproteinase 7 (matrilysin, uterine)	MMP7	Colorectal Cancer
206756_at	carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 7	CHST7	Colorectal Cancer
202003_s_at	acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)	ACAA2	Colorectal Cancer
218755_at	kinesin family member 20A	KIF20A	Colorectal Cancer
202545_at	protein kinase C, delta	PRKCD	Colorectal Cancer
218555_at	anaphase-promoting complex subunit 2	ANAPC2	Colorectal Cancer
205506_at	villin 1	VIL1	Colorectal Cancer
220974_x_at	similar to rat tricarboxylate carrier-like protein	BA108L7.2	Colorectal Cancer
206130_s_at	asialoglycoprotein receptor 2	ASGR2	Colorectal Cancer
205025_at	GLI-Kruppel family member HKR3	HKR3	Colorectal Cancer
219278_at	mitogen-activated protein kinase kinase kinase 6	MAP3K6	Colorectal Cancer
219452_at	putative dipeptidase	LOC64174	Colorectal Cancer
205997_at	a disintegrin and metalloproteinase domain 28	ADAM28	Colorectal Cancer
206074_s_at	high mobility group AT-hook 1	HMGA1	Colorectal Cancer
219386_s_at	B lymphocyte activator macrophage expressed	BLAME	Colorectal Cancer
201082_s_at	dynactin 1 (p150, glued homolog, Drosophila)	DCTN1	Colorectal Cancer

213746_s_at	filamin A, alpha (actin binding protein 280)	FLNA	Colorectal Cancer
209821_at	DVS27-related protein	DVS27	Colorectal Cancer
212871_at	mitogen-activated protein kinase-activated protein kinase 5	MAPKAPK5	Colorectal Cancer
209267_s_at	BCG-induced gene in monocytes, clone 103	BIGM103	Colorectal Cancer
201880_at	ariadne homolog, ubiquitin-conjugating enzyme E2 binding protein, 1 (Drosophila)	ARIH1	Colorectal Cancer
201215_at	plastin 3 (T isoform)	PLS3	Colorectal Cancer
220748_s_at	LDL induced EC protein	LOC51157	Colorectal Cancer
201620_at	membrane-bound transcription factor protease, site 1	MBTPS1	Colorectal Cancer
204857_at	MAD1 mitotic arrest deficient-like 1 (yeast)	MAD1L1	Colorectal Cancer
216942_s_at	CD58 antigen, (lymphocyte function-associated antigen 3)	CD58	Colorectal Cancer
206120_at	CD33 antigen (gp67)	CD33	Colorectal Cancer
218831_s_at	Fc fragment of IgG, receptor, transporter, alpha	FCGRT	Colorectal Cancer
217789_at	sorting nexin 6	SNX6	Colorectal Cancer
210889_s_at	Fc fragment of IgG, low affinity IIb, receptor for (CD32)	FCGR2B	Colorectal Cancer
203979_at	cytochrome P450, family 27, subfamily A, polypeptide 1	CYP27A1	Colorectal Cancer
205418_at	feline sarcoma oncogene	FES	Colorectal Cancer
204233_s_at	choline kinase	CHK	Colorectal Cancer
219613_s_at	sirtuin (silent mating type information regulation 2 homolog) 6 (S. cerevisiae)	SIRT6	Colorectal Cancer
204742_s_at	androgen-induced proliferation inhibitor	APRIN	Colorectal Cancer
202831_at	glutathione peroxidase 2 (gastrointestinal)	GPX2	Colorectal Cancer
201389_at	integrin, alpha 5 (fibronectin receptor, alpha polypeptide)	ITGA5	Colorectal Cancer
203996_s_at	chromosome 21 open reading frame 2	C21orf2	Colorectal Cancer
211200_s_at	Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog	FGR	Colorectal Cancer
219593_at	peptide transporter 3	PHT2	Colorectal Cancer
212858_at	hypothetical protein FLJ30002	FLJ30002	Colorectal Cancer
206682_at	macrophage lectin 2 (calcium dependent)	HML2	Colorectal Cancer
210640_s_at	G protein-coupled receptor 30	GPR30	Colorectal Cancer
220307_at	natural killer cell receptor 2B4	CD244	Colorectal Cancer
209615_s_at	p21/Cdc42/Rac1-activated kinase 1 (STE20	PAK1	Colorectal Cancer

	homolog, yeast)		
41160_at	methyl-CpG binding domain protein 3	MBD3	Colorectal Cancer
206206_at	lymphocyte antigen 64 homolog, radioprotective 105kDa (mouse)	LY64	Colorectal Cancer
220519_s_at	lens intrinsic membrane protein 2, 19kDa	LIM2	Colorectal Cancer
220068_at	pre-B lymphocyte gene 3	VPREB3	Colorectal Cancer
215273_s_at	transcriptional adaptor 3 (NGG1 homolog, yeast)-like	TADA3L	Colorectal Cancer
203903_s_at	hephaestin	HEPH	Colorectal Cancer
219911_s_at	solute carrier family 21 (organic anion transporter), member 12	SLC21A12	Colorectal Cancer
219366_at	apoptosis, caspase activation inhibitor	AVEN	Colorectal Cancer
218673_s_at	ubiquitin activating enzyme E1-like protein	GSA7	Colorectal Cancer
218345_at	hepatocellular carcinoma-associated antigen 112	HCA112	Colorectal Cancer
202496_at	autoantigen	RCD-8	Colorectal Cancer
217923_at	PEF protein with a long N-terminal hydrophobic domain (pefin)	PEF	Colorectal Cancer
216199_s_at	mitogen-activated protein kinase kinase kinase 4	MAP3K4	Colorectal Cancer
35617_at	mitogen-activated protein kinase 7	MAPK7	Colorectal Cancer
203043_at	Ac-like transposable element	ALTE	Colorectal Cancer
205048_s_at	phosphoserine phosphatase-like	PSPHL	Colorectal Cancer
203938_s_at	TATA box binding protein (TBP)-associated factor, RNA polymerase I, C, 110kDa	TAF1C	Colorectal Cancer
206398_s_at	CD19 antigen	CD19	Colorectal Cancer
220762_s_at	guanine nucleotide binding protein (G protein), beta polypeptide 1-like	GNB1L	Colorectal Cancer
205547_s_at	transgelin	TAGLN	Colorectal Cancer
204272_at	lectin, galactoside-binding, soluble, 4 (galectin 4)	LGALS4	Colorectal Cancer
204790_at	MAD, mothers against decapentaplegic homolog 7 (Drosophila)	MADH7	Colorectal Cancer
208070_s_at	REV3-like, catalytic subunit of DNA polymerase zeta (yeast)	REV3L	Colorectal Cancer
202953_at	complement component 1, q subcomponent, beta polypeptide	C1QB	Colorectal Cancer
1053_at	replication factor C (activator 1) 2, 40kDa	RFC2	Breast and Prostate Cancers
200636_s_at	protein tyrosine phosphatase, receptor type, F	PTPRF	Breast and Prostate Cancers

200878_at	Homo sapiens clone 23698 mRNA sequence		Breast and Prostate Cancers
201066_at	cytochrome c-1	CYC1	Breast and Prostate Cancers
201212_at	legumain	LGMN	Breast and Prostate Cancers
201289_at	cysteine-rich, angiogenic inducer, 61	CYR61	Breast and Prostate Cancers
201388_at	proteasome (prosome, macropain) 26S subunit, non-ATPase, 3	PSMD3	Breast and Prostate Cancers
201428_at	claudin 4	CLDN4	Breast and Prostate Cancers
201829_at	neuroepithelial cell transforming gene 1	NET1	Breast and Prostate Cancers
201946_s_at	chaperonin containing TCP1, subunit 2 (beta)	CCT2	Breast and Prostate Cancers
202023_at	ephrin-A1	EFNA1	Breast and Prostate Cancers
202376_at	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	SERPINA3	Breast and Prostate Cancers
202562_s_at	chromosome 14 open reading frame 1	C14orf1	Breast and Prostate Cancers
203130_s_at	kinesin family member 5C	KIF5C	Breast and Prostate Cancers
203213_at	cell division cycle 2, G1 to S and G2 to M	CDC2	Breast and Prostate Cancers
204199_at	Ral guanine nucleotide exchange factor RalGPS1A	RALGPS1A	Breast and Prostate Cancers
204547_at	RAB40B, member RAS oncogene family	RAB40B	Breast and Prostate Cancers
204559_s_at	LSM7 homolog, U6 small nuclear RNA associated (S. cerevisiae)	LSM7	Breast and Prostate Cancers
205420_at	peroxisomal biogenesis factor 7	PEX7	Breast and Prostate Cancers
205542_at	six transmembrane epithelial antigen of the prostate	STEAP	Breast and Prostate Cancers
205890_s_at	ubiquitin D	UBD	Breast and Prostate Cancers
206858_s_at	homeo box C6	HOXC6	Breast and Prostate Cancers
209114_at	tetraspan 1	TSPAN-1	Breast and Prostate Cancers
209487_at	RNA binding protein with multiple splicing	RBPMS	Breast and Prostate Cancers
211941_s_at	prostatic binding protein	PBP	Breast and

			Prostate Cancers
213441_x_at	prostate epithelium-specific Ets transcription factor	PDEF	Breast and Prostate Cancers
214375_at	PTPRF interacting protein, binding protein 1 (liprin beta 1)	PPFIBP1	Breast and Prostate Cancers
217716_s_at	protein transport protein SEC61 alpha subunit isoform 1	SEC61A1	Breast and Prostate Cancers
217973_at	dicarbonyl/L-xylulose reductase	DCXR	Breast and Prostate Cancers
218481_at	exosome component Rrp46	RRP46	Breast and Prostate Cancers
219373_at	dolichyl-phosphate mannosyltransferase polypeptide 3	DPM3	Breast and Prostate Cancers
221521_s_at	HSPC037 protein	LOC51659	Breast and Prostate Cancers
201876_at	paraoxonase 2	PON2	Breast and Colorectal Cancers
202148_s_at	pyrroline-5-carboxylate reductase 1	PYCR1	Breast and Colorectal Cancers
202936_s_at	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	SOX9	Breast and Colorectal Cancers
203108_at	retinoic acid induced 3	RAI3	Breast and Colorectal Cancers
203453_at	sodium channel, nonvoltage-gated 1 alpha	SCNN1A	Breast and Colorectal Cancers
203757_s_at	carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)	CEACAM6	Breast and Colorectal Cancers
203767_s_at	steroid sulfatase (microsomal), arylsulfatase C, isozyme S	STS	Breast and Colorectal Cancers
204517_at	peptidylprolyl isomerase C (cyclophilin C)	PPIC	Breast and Colorectal Cancers
204641_at	NIMA (never in mitosis gene a)-related kinase 2	NEK2	Breast and Colorectal Cancers
205260_s_at	acylphosphatase 1, erythrocyte (common) type	ACYP1	Breast and Colorectal Cancers
205774_at	coagulation factor XII (Hageman factor)	F12	Breast and Colorectal Cancers
208029_s_at	lysosomal associated protein transmembrane 4 beta	LAPTM4B	Breast and Colorectal Cancers
208161_s_at	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	ABCC3	Breast and Colorectal Cancers
209173_at	anterior gradient 2 homolog (Xenepus laevis)	AGR2	Breast and Colorectal Cancers
218002_s_at	chemokine (C-X-C motif) ligand 14	CXCL14	Breast and

			Colorectal Cancers
218459_at	ATP-dependant interferon responsive	ADIR	Breast and Colorectal Cancers
218670_at	pseudouridylate synthase 1	PUS1	Breast and Colorectal Cancers
203824_at	transmembrane 4 superfamily member 3	TM4SF3	Prostate and Colorectal Cancers
204137_at	transmembrane 7 superfamily member 1 (upregulated in kidney)	TM7SF1	Prostate and Colorectal Cancers
205987_at	CD1C antigen, c polypeptide	CD1C	Prostate and Colorectal Cancers
206308_at	DNA (cytosine-5-)-methyltransferase 2	DNMT2	Prostate and Colorectal Cancers
207541_s_at	polymyositis/scleroderma autoantigen 2, 100kDa	PMSCL2	Prostate and Colorectal Cancers
208631_s_at	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit	HADHA	Prostate and Colorectal Cancers
209620_s_at	ATP-binding cassette, sub-family B (MDR/TAP), member 7	ABCB7	Prostate and Colorectal Cancers
209785_s_at	phospholipase A2, group IVC (cytosolic, calcium-independent)	PLA2G4C	Prostate and Colorectal Cancers
210448_s_at	purinergic receptor P2X, ligand-gated ion channel, 5	P2RX5	Prostate and Colorectal Cancers
221004_s_at	integral membrane protein 2C	ITM2C	Prostate and Colorectal Cancers
201113_at	Tu translation elongation factor, mitochondrial	TUFM	Breast, Prostate and Colorectal Cancers
201260_s_at	synaptophysin-like protein	SYPL	Breast, Prostate and Colorectal Cancers
201263_at	threonyl-tRNA synthetase	TARS	Breast, Prostate and Colorectal Cancers
201415_at	glutathione synthetase	GSS	Breast, Prostate and Colorectal Cancers
201417_at	Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 1977059		Breast, Prostate and Colorectal Cancers
201427_s_at	selenoprotein P, plasma, 1	SEPP1	Breast, Prostate and Colorectal Cancers
201596_x_at	keratin 18	KRT18	Breast, Prostate and Colorectal Cancers
201650_at	keratin 19	KRT19	Breast, Prostate and Colorectal Cancers
201839_s_at	tumor-associated calcium signal transducer 1	TACSTD1	Breast, Prostate and Colorectal Cancers
201892_s_at	IMP (inosine monophosphate) dehydrogenase	IMPDH2	Breast, Prostate

	2		and Colorectal Cancers
202286_s_at	tumor-associated calcium signal transducer 2	TACSTD2	Breast, Prostate and Colorectal Cancers
202401_s_at	serum response factor (c-fos serum response element-binding transcription factor)	SRF	Breast, Prostate and Colorectal Cancers
202597_at	ESTs, Weakly similar to YYY1_HUMAN Very very hypothetical protein RMSA-1 [H.sapiens]		Breast, Prostate and Colorectal Cancers
202598_at	S100 calcium binding protein A13	S100A13	Breast, Prostate and Colorectal Cancers
202705_at	cyclin B2	CCNB2	Breast, Prostate and Colorectal Cancers
202768_at	FBJ murine osteosarcoma viral oncogene homolog B	FOSB	Breast, Prostate and Colorectal Cancers
202942_at	electron-transfer-flavoprotein, beta polypeptide	ETFB	Breast, Prostate and Colorectal Cancers
203038_at	protein tyrosine phosphatase, receptor type, K	PTPRK	Breast, Prostate and Colorectal Cancers
203152_at	mitochondrial ribosomal protein L40	MRPL40	Breast, Prostate and Colorectal Cancers
203190_at	NADH dehydrogenase (ubiquinone) Fe-S protein 8, 23kDa (NADH-coenzyme Q reductase)	NDUFS8	Breast, Prostate and Colorectal Cancers
203478_at	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 1, 6kDa	NDUFC1	Breast, Prostate and Colorectal Cancers
203917_at	coxsackie virus and adenovirus receptor	CXADR	Breast, Prostate and Colorectal Cancers
204170_s_at	CDC28 protein kinase regulatory subunit 2	CKS2	Breast, Prostate and Colorectal Cancers
204623_at	trefoil factor 3 (intestinal)	TFF3	Breast, Prostate and Colorectal Cancers
206683_at	zinc finger protein 165	ZNF165	Breast, Prostate and Colorectal Cancers
207076_s_at	argininosuccinate synthetase	ASS	Breast, Prostate and Colorectal Cancers
208794_s_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	SMARCA4	Breast, Prostate and Colorectal Cancers
208862_s_at	catenin (cadherin-associated protein), delta 1	CTNND1	Breast, Prostate and Colorectal Cancers
209094_at	dimethylarginine dimethylaminohydrolase 1	DDAH1	Breast, Prostate and Colorectal Cancers
209486_at	disrupter of silencing 10	SAS10	Breast, Prostate and Colorectal Cancers
209605_at	thiosulfate sulfurtransferase (rhodanese)	TST	Breast, Prostate and Colorectal Cancers

209731_at	nth endonuclease III-like 1 (E. coli)	NTHL1	Breast, Prostate and Colorectal Cancers
210512_s_at	vascular endothelial growth factor	VEGF	Breast, Prostate and Colorectal Cancers
212429_s_at	general transcription factor IIIC, polypeptide 2, beta 110kDa	GTF3C2	Breast, Prostate and Colorectal Cancers
214096_s_at	serine hydroxymethyltransferase 2 (mitochondrial)	SHMT2	Breast, Prostate and Colorectal Cancers
215171_s_at	translocase of inner mitochondrial membrane 17 homolog A (yeast)	TIMM17A	Breast, Prostate and Colorectal Cancers
217744_s_at	p53-induced protein PIGPC1	PIGPC1	Breast, Prostate and Colorectal Cancers
217772_s_at	mitochondrial carrier homolog 2	MTCH2	Breast, Prostate and Colorectal Cancers
217901_at	Homo sapiens, clone IMAGE:4047062, mRNA		Breast, Prostate and Colorectal Cancers
218009_s_at	rotein regulator of cytokinesis 1	PRC1	Breast, Prostate and Colorectal Cancers
218188_s_at	translocase of inner mitochondrial membrane 13 homolog (yeast)	TIMM13	Breast, Prostate and Colorectal Cancers
218436_at	endoplasmic reticulum chaperone SIL1, homolog of yeast	SIL1	Breast, Prostate and Colorectal Cancers
219244_s_at	mitochondrial ribosomal protein L46	MRPL46	Breast, Prostate and Colorectal Cancers

Table 2 condenses the number of these selected genes to a number that is easily used in rapid screening. Gene numbers for breast cancer (10), prostate (7), colorectal (7), and combinations thereof (7) showed the most prominent signal-to-noise separation and, thus, were appropriate in number and type for profile analysis. These combinations provide a collection of genes that could have diagnostic/prognostic significance in the treatment of cancer.

Table 2: Reduction in the number of genes to limit each panel to a workable number for rapid screening.

			Colorectal Cancer n=40	Colorectal Cancer n=40	Prostate Cancer n=42	Prostate Cancer n=42	Breast Cancer n=13	Breast Cancer n=13	Cancer Free Donors n=56	Cancer Free Donors n=56
Cancer Ty	Gene Name	Measure Gene Symbol	Mean copy number of transcripts	% positive samples (with number of transcript copies greater than 95% of all normal donors)	Mean copy number of transcripts	% positive samples (with number of transcript copies greater than 95% of all normal donors)	Mean copy number of transcripts	% positive samples (with number of transcript copies greater than 95% of all normal donors)	Mean copy number of transcripts	% positive samples (with number of transcript copies greater than 95% of all normal donors)
Control	actin, beta	ACTB	20626	25	21315	26	49278	77	12347	4
Control	ribosomal protein S27a	RPS27A	14694	10	13503	7	20880	15	11656	5
Combined	Keratin 19	KRT19	104	58	48	62	751	77	1	4
Combined	anterior gradient 2 homolog (Xenepus laevis)	AGR2	338	30	681	38	1078	54	3	2
Combined	trefoil factor 3 (intestinal)	TFF3	1467	13	1077	12	4534	23	141	4
Combined	endoplasmic reticulum chaperone SIL1, homolog of yeast	SIL1	142	10	140	21	372	54	59	4
Combined	beta-site APP-cleaving enzyme 2	BACE2	144	0	277	0	187	0	272	5
Combined	immature colon carcinoma transcript 1	ICT1	555	8	680	19	1007	46	380	7
Combined	thiosulfate sulfurtransferase (rhodanese)	TST	1779	35	1615	43	2256	54	587	4
Breast	mammoglobin 1	MGB1	6	30	2	10	7732	62	1	2
Breast	secretoglobin, family 2A, member 1	SCGB2A1	0	13	1	26	46	46	0	0
Breast	S100 calcium binding protein A7 (psoriasin 1)	S100A7	0	0	3	10	308	15	1	2
Breast	monoamine oxidase B	CALML5	0	0	5	21	156	46	0	0
Breast	solute carrier family 2 (facilitated glucose transporter), member 10	TFAPB2	0	0	0	2	13	31	0	2
Breast	transcription factor AP-2 beta (activating enhancer binding protein 2 beta)	ESR1	21	3	28	7	518	31	24	5
Breast	prolactin-induced protein	SLC2A10	7	5	20	12	273	31	6	2
Breast	estrogen receptor 1	PIP	2	0	51	7	755	46	5	2
Breast	calmodulin-like skin protein	CYP4B1	3	13	34	21	13	31	0	2
Breast	cytochrome P450, family 4, subfamily B, polypeptide 1	MAOB	135	15	60	5	291	38	16	2
Prostate	prostate specific antigen	PSA	92	3	30554	60	1	0	4	2
Prostate	kallikrein 2, prostatic	KLK2	0	5	82	50	0	0	0	2
Prostate	microseminoprotein, beta-	NPY	1	3	477	36	0	0	1	7
Prostate	neuropeptide Y	MSMB	0	5	125	55	1	23	0	9
Prostate	hepsin	DDC	3	33	28	31	50	8	0	5
Prostate	dopa decarboxylase (aromatic L-amino acid decarboxylase)	AR	2	13	107	50	37	38	1	4
Prostate	androgen receptor	HPN	1	3	47	43	27	54	1	2
Colorectal	keratin 20	CK20	93	50	5	29	1	15	0	5
Colorectal	carcinoembryonic antigen-related cell adhesion molecule 5	CEA	394	45	1	10	145	54	0	2
Colorectal	macrophage receptor with collagenous structure	ADAM28	302	3	656	7	727	8	801	5
Colorectal	fatty acid binding protein 1, liver	ASGR	1781	20	1356	31	4712	77	474	2
Colorectal	villin 1	FABP1	280	50	16	19	1	15	1	5
Colorectal	asialoglycoprotein receptor 2	MARCO	2607	35	3050	43	4007	69	542	2
Colorectal	a disintegrin and metalloproteinase domain 28	VIL1	55	23	10	10	62	31	6	4

EXAMPLE 4

Microarray Expression Analysis in Genes Detectable after CTC Depletion

Analysis of genes detected prior to depletion of the WBC fraction and after subtraction of the WBC resulted in sets of genes substantially attenuated in the depleted portion. Gene sets were the CTC levels are at least 3 fold greater than the CTC depleted WBC detectable signal are shown in Table 3. As with Example 3, the same patient groups (breast, prostate, colorectal) were compared.

Table 3: Genetic profile where at least a 3 fold reduction in the individual gene signal was detected in the WBC-depleted portion.

Affy ID	Gene Name	Gene Symbol	Predicted Gene Expression CTC Specificity
206378_at	secretoglobin, family 2A, member 2	SCGB2A2	Breast cancer
208451_s_at	complement component 4B	C4B	Breast cancer
204653_at	transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)	TFAP2A	Breast cancer
201599_at	ornithine aminotransferase (gyrate atrophy)	OAT	Breast cancer
202704_at	transducer of ERBB2, 1	TOB1	Breast cancer
204351_at	S100 calcium binding protein P	S100P	Breast cancer
204404_at	solute carrier family 12 (sodium/potassium/chloride transporters), member 2	SLC12A2	Breast cancer
202118_s_at	copine III	CPNE3	Breast cancer
203476_at	trophoblast glycoprotein	TPBG	Breast cancer
200800_s_at	heat shock 70kDa protein 1A	HSPA1A	Breast cancer
217722_s_at	mesenchymal stem cell protein DSC92	NEUGRIN	Breast cancer
205251_at	period homolog 2 (Drosophila)	PER2	Breast cancer
200830_at	proteasome (prosome, macropain) 26S subunit, non-ATPase, 2	PSMD2	Breast cancer
36936_at	tissue specific transplantation antigen P35B	TSTA3	Breast cancer
218735_s_at	zinc finger protein	AF020591	Breast cancer
39729_at	peroxiredoxin 2	PRDX2	Breast cancer
208949_s_at	lectin, galactoside-binding, soluble, 3 (galectin 3)	LGALS3	Breast cancer
209449_at	LSM2 homolog, U6 small nuclear RNA associated (S. cerevisiae)	LSM2	Breast cancer
209510_at	patched related protein translocated in renal cancer	TRC8	Breast cancer
212461_at	ornithine decarboxylase antizyme inhibitor	OAZIN	Breast cancer
212652_s_at	sorting nexin 4	SNX4	Breast cancer
218224_at	paraneoplastic antigen MA1	PNMA1	Breast cancer
218356_at	FtsJ homolog 2 (E. coli)	FTSJ2	Breast cancer
200048_s_at	jumping translocation breakpoint	JTB	Breast cancer

209706_at	NK3 transcription factor related, locus 1 (Drosophila)	NKX3-1	Prostate cancer
202429_s_at	protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform (calcineurin A alpha)	PPP3CA	Prostate cancer
208737_at	ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G isoform 1	ATP6V1G1	Prostate cancer
213655_at	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	YWHAE	Prostate cancer
205483_s_at	interferon, alpha-inducible protein (clone IFI-15K)	G1P2	Prostate cancer
210338_s_at	heat shock 70kDa protein 8	HSPA8	Prostate cancer
214290_s_at	histone 2, H2aa	HIST2H2AA	Prostate cancer
219117_s_at	FK506 binding protein 11, 19 kDa	FKBP11	Prostate cancer
201138_s_at	Sjogren syndrome antigen B (autoantigen La)	SSB	Prostate cancer
201530_x_at	eukaryotic translation initiation factor 4A, isoform 1	EIF4A1	Prostate cancer
202241_at	phosphoprotein regulated by mitogenic pathways	C8FW	Prostate cancer
208756_at	eukaryotic translation initiation factor 3, subunit 2 beta, 36kDa	EIF3S2	Prostate cancer
209250_at	degenerative spermatocyte homolog, lipid desaturase (Drosophila)	DEGS	Prostate cancer
218206_x_at	SCAN domain containing 1	SCAND1	Prostate cancer
218250_s_at	CCR4-NOT transcription complex, subunit 7	CNOT7	Prostate cancer
210378_s_at	Sjogren's syndrome nuclear autoantigen 1	SSNA1	Prostate cancer
202211_at	ADP-ribosylation factor GTPase activating protein 3	ARFGAP3	Prostate cancer
200083_at	ubiquitin specific protease 22	USP22	Prostate cancer
201197_at	S-adenosylmethionine decarboxylase 1	AMD1	Prostate cancer
201317_s_at	proteasome (prosome, macropain) subunit, alpha type, 2	PSMA2	Prostate cancer
210835_s_at	C-terminal binding protein 2	CTBP2	Prostate cancer
201730_s_at	translocated promoter region (to activated MET oncogene)	TPR	Prostate cancer
201909_at	ribosomal protein S4, Y-linked	RPS4Y	Prostate cancer
201953_at	calcium and integrin binding 1 (calmyrin)	CIB1	Prostate cancer
201968_s_at	phosphoglucomutase 1	PGM1	Prostate cancer
203373_at	suppressor of cytokine signaling 2	SOCS2	Prostate cancer
202168_at	TAF9 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 32kDa	TAF9	Prostate cancer
209303_at	NADH dehydrogenase (ubiquinone) Fe-S protein 4, 18kDa (NADH-coenzyme Q reductase)	NDUFS4	Prostate cancer
205347_s_at	thymosin, beta, identified in neuroblastoma cells	TMSNB	Prostate cancer
218003_s_at	FK506 binding protein 3, 25kDa	FKBP3	Prostate cancer
204219_s_at	proteasome (prosome, macropain) 26S subunit, ATPase, 1	PSMC1	Prostate cancer
200039_s_at	proteasome (prosome, macropain) subunit, beta type, 2	PSMB2	Prostate cancer
218671_s_at	ATPase inhibitory factor 1	ATPIF1	Prostate cancer
218357_s_at	translocase of inner mitochondrial membrane 8 homolog B (yeast)	TIMM8B	Prostate cancer
208841_s_at	Ras-GTPase activating protein SH3 domain-binding protein 2	G3BP2	Prostate cancer
201999_s_at	t-complex-associated-testis-expressed 1-like 1	TCTEL1	Prostate cancer
209619_at	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	CD74	Colorectal cancer

201506_at	transforming growth factor, beta-induced, 68kDa	TGFBI	Colorectal cancer
206493_at	integrin, alpha 2b (platelet glycoprotein IIb of IIb/IIIa complex, antigen CD41B)	ITGA2B	Colorectal cancer
208450_at	lectin, galactoside-binding, soluble, 2 (galectin 2)	LGALS2	Colorectal cancer
201360_at	cystatin C (amyloid angiopathy and cerebral hemorrhage)	CST3	Colorectal cancer
218649_x_at	serologically defined colon cancer antigen 1	SDCCAG1	Colorectal cancer
200743_s_at	ceroid-lipofuscinosis, neuronal 2, late infantile (Jansky-Bielschowsky disease)	CLN2	Colorectal cancer
204158_s_at	T-cell, immune regulator 1, ATPase, H+ transporting, lysosomal V0 protein a isoform 3	TCIRG1	Colorectal cancer
205241_at	SCO cytochrome oxidase deficient homolog 2 (yeast)	SCO2	Colorectal cancer
207857_at	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 2	LILRA2	Colorectal cancer
202625_at	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	LYN	Colorectal cancer
201795_at	lamin B receptor	LBR	Colorectal cancer
209248_at	growth hormone inducible transmembrane protein	GHITM	Colorectal cancer
218559_s_at	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)	MAFB	Colorectal cancer
210146_x_at	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2	LILRB2	Colorectal cancer
200945_s_at	yeast Sec31p homolog	KIAA0905	Colorectal cancer
203778_at	mannosidase, beta A, lysosomal	MANBA	Colorectal cancer
203645_s_at	CD163 antigen	CD163	Colorectal cancer
203508_at	tumor necrosis factor receptor superfamily, member 1B	TNFRSF1B	Colorectal cancer
203459_s_at	vacuolar protein sorting 16 (yeast)	VPS16	Colorectal cancer
203317_at	SEC7 homolog	TIC	Colorectal cancer
201931_at	electron-transfer-flavoprotein, alpha polypeptide (glutaric aciduria II)	ETFA	Colorectal cancer
202794_at	inositol polyphosphate-1-phosphatase	INPP1	Colorectal cancer
201012_at	annexin A1	ANXA1	Colorectal cancer
202413_s_at	ubiquitin specific protease 1	USP1	Colorectal cancer
202295_s_at	cathepsin H	CTSH	Colorectal cancer
201163_s_at	insulin-like growth factor binding protein 7	IGFBP7	Colorectal cancer
201186_at	low density lipoprotein-related protein-associated protein 1 (alpha-2-macroglobulin receptor-associated protein 1)	LRPAP1	Colorectal cancer
201331_s_at	signal transducer and activator of transcription 6, interleukin-4 induced	STAT6	Colorectal cancer
200762_at	dihydropyrimidinase-like 2	DPYSL2	Colorectal cancer
208792_s_at	clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J)	CLU	Colorectal cancer
218088_s_at	Rag C protein	GTR2	Colorectal cancer
217843_s_at	HSPC126 protein	HSPC126	Colorectal cancer
217751_at	glutathione S-transferase subunit 13 homolog	LOC51064	Colorectal cancer
213566_at	ribonuclease, RNase A family, k6	RNASE6	Colorectal cancer
212501_at	CCAAT/enhancer binding protein (C/EBP), beta	CEBPB	Colorectal cancer
212360_at	adenosine monophosphate deaminase 2 (isoform L)	AMPD2	Colorectal cancer
211284_s_at	granulin	GRN	Colorectal cancer

209786_at	high mobility group nucleosomal binding domain 4	HMGN4	Colorectal cancer
204759_at	chromosome condensation 1-like	CHC1L	Colorectal cancer
209037_s_at	EH-domain containing 1	EHD1	Colorectal cancer
204099_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3	SMARCD3	Colorectal cancer
208700_s_at	transketolase (Wernicke-Korsakoff syndrome)	TKT	Colorectal cancer
208683_at	calpain 2, (m/II) large subunit	CAPN2	Colorectal cancer
208610_s_at	serine/arginine repetitive matrix 2	SRRM2	Colorectal cancer
208146_s_at	carboxypeptidase, vitellogenic-like	CPVL	Colorectal cancer
207785_s_at	H-2K binding factor-2	KBF2	Colorectal cancer
221059_s_at	carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 6	CHST6	Colorectal cancer
205898_at	chemokine (C-X3-C motif) receptor 1	CX3CR1	Colorectal cancer
200600_at	moesin	MSN	Colorectal cancer
204276_at	thymidine kinase 2, mitochondrial	TK2	Colorectal cancer
209166_s_at	mannosidase, alpha, class 2B, member 1	MAN2B1	Colorectal cancer
200094_s_at	eukaryotic translation elongation factor 2	EEF2	Colorectal and prostate cancers
200788_s_at	phosphoprotein enriched in astrocytes 15	PEA15	Colorectal and prostate cancers
208095_s_at	calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma	CAMK2G	Colorectal and prostate cancers
218467_at	hepatocellular carcinoma susceptibility protein	HCCA3	Colorectal and prostate cancers
200875_s_at	nucleolar protein 5A (56kDa with KKE/D repeat)	NOL5A	Colorectal and breast cancers
201590_x_at	annexin A2	ANXA2	Colorectal and breast cancers
208691_at	transferrin receptor (p90, CD71)	TFRC	Colorectal and breast cancers
211285_s_at	ubiquitin protein ligase E3A (human papilloma virus E6-associated protein, Angelman syndrome)	UBE3A	Colorectal and breast cancers
216520_s_at	tumor protein, translationally-controlled 1	TPT1	Colorectal and breast cancers
218269_at	putative ribonuclease III	RNASE3L	Colorectal and breast cancers
221841_s_at	Homo sapiens cDNA FLJ38575 fis, clone HCHON2007046.		Colorectal and breast cancers
221989_at	ribosomal protein L10	RPL10	Colorectal and breast cancers
200013_at	dipeptidylpeptidase 7	DPP7	Breast and prostate cancers
200024_at	ribosomal protein S5	RPS5	Breast and prostate cancers
200064_at	heat shock 90kDa protein 1, beta	HSPCB	Breast and prostate cancers
200614_at	clathrin, heavy polypeptide (Hc)	CLTC	Breast and prostate cancers
200652_at	signal sequence receptor, beta (translocon-associated protein beta)	SSR2	Breast and prostate cancers
200658_s_at	prohibitin	PHB	Breast and prostate cancers
200716_x_at	ribosomal protein L13a	RPL13A	Breast and prostate cancers
200823_x_at	ribosomal protein L29	RPL29	Breast and prostate cancers
200936_at	ribosomal protein L8	RPL8	Breast and prostate cancers
200937_s_at	ribosomal protein L5	RPL5	Breast and prostate cancers

201119_s_at	cytochrome c oxidase subunit VIII	COX8	Breast and prostate cancers
201517_at	nuclear cap binding protein subunit 2, 20kDa	NCBP2	Breast and prostate cancers
201577_at	non-metastatic cells 1, protein (NM23A) expressed in	NME1	Breast and prostate cancers
202324_s_at	golgi complex associated protein 1, 60kDa	GOCAP1	Breast and prostate cancers
205807_s_at	tuftelin 1	TUFT1	Breast and prostate cancers
208612_at	glucose regulated protein, 58kDa	GRP58	Breast and prostate cancers
208886_at	H1 histone family, member 0	H1F0	Breast and prostate cancers
210213_s_at	integrin beta 4 binding protein	ITGB4BP	Breast and prostate cancers
211937_at	eukaryotic translation initiation factor 4B	EIF4B	Breast and prostate cancers
212581_x_at	glyceraldehyde-3-phosphate dehydrogenase	GAPD	Breast and prostate cancers
213757_at	eukaryotic translation initiation factor 5A	EIF5A	Breast and prostate cancers
213897_s_at	mitochondrial ribosomal protein L23	MRPL23	Breast and prostate cancers
214167_s_at	ribosomal protein, large, P0	RPLP0	Breast and prostate cancers
215726_s_at	cytochrome b-5	CYB5	Breast and prostate cancers
217802_s_at	similar to rat nuclear ubiquitous casein kinase 2	NUCKS	Breast and prostate cancers
217871_s_at	macrophage migration inhibitory factor (glycosylation-inhibiting factor)	MIF	Breast and prostate cancers
218200_s_at	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2, 8kDa	NDUFB2	Breast and prostate cancers
218213_s_at	chromosome 11 open reading frame 10	C11orf10	Breast and prostate cancers
218226_s_at	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 4, 15kDa	NDUFB4	Breast and prostate cancers
218253_s_at	ligatin	LGTN	Breast and prostate cancers
221488_s_at	divalent cation tolerant protein CUTA	LOC51596	Breast and prostate cancers
36711_at	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	MAFF	Breast and prostate cancers
39248_at	aquaporin 3	AQP3	Breast and prostate cancers
200063_s_at	nucleophosmin (nucleolar phosphoprotein B23, numatrin)	NPM1	Breast, colorectal and prostate cancers
200093_s_at	histidine triad nucleotide binding protein 1	HINT1	Breast, colorectal and prostate cancers
200599_s_at	tumor rejection antigen (gp96) 1	TRA1	Breast, colorectal and prostate cancers
200642_at	superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	SOD1	Breast, colorectal and prostate cancers
200651_at	guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1	GNB2L1	Breast, colorectal and prostate cancers

200657_at	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5	SLC25A5	Breast, colorectal and prostate cancers
200807_s_at	heat shock 60kDa protein 1 (chaperonin)	HSPD1	Breast, colorectal and prostate cancers
200818_at	ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit (oligomycin sensitivity conferring protein)	ATP5O	Breast, colorectal and prostate cancers
200826_at	small nuclear ribonucleoprotein D2 polypeptide 16.5kDa	SNRPD2	Breast, colorectal and prostate cancers
200858_s_at	ribosomal protein S8	RPS8	Breast, colorectal and prostate cancers
200877_at	chaperonin containing TCP1, subunit 4 (delta)	CCT4	Breast, colorectal and prostate cancers
200910_at	chaperonin containing TCP1, subunit 3 (gamma)	CCT3	Breast, colorectal and prostate cancers
201022_s_at	destrin (actin depolymerizing factor)	DSTN	Breast, colorectal and prostate cancers
201049_s_at	ribosomal protein S18	RPS18	Breast, colorectal and prostate cancers
201077_s_at	NHP2 non-histone chromosome protein 2-like 1 (S. cerevisiae)	NHP2L1	Breast, colorectal and prostate cancers
201201_at	cystatin B (stefin B)	CSTB	Breast, colorectal and prostate cancers
201994_at	mortality factor 4 like 2	MORF4L2	Breast, colorectal and prostate cancers
202282_at	hydroxyacyl-Coenzyme A dehydrogenase, type II	HADH2	Breast, colorectal and prostate cancers
202428_x_at	diazepam binding inhibitor (GABA receptor modulator, acyl-Coenzyme A binding protein)	DBI	Breast, colorectal and prostate cancers
202475_at	seven transmembrane domain protein	NIFIE14	Breast, colorectal and prostate cancers
203316_s_at	small nuclear ribonucleoprotein polypeptide E	SNRPE	Breast, colorectal and prostate cancers
205133_s_at	heat shock 10kDa protein 1 (chaperonin 10)	HSPE1	Breast, colorectal and prostate cancers
208697_s_at	eukaryotic translation initiation factor 3, subunit 6 48kDa	EIF3S6	Breast, colorectal and prostate cancers
208787_at	mitochondrial ribosomal protein L3	MRPL3	Breast, colorectal and prostate cancers
208905_at	cytochrome c, somatic	CYCS	Breast, colorectal and prostate cancers
209058_at	endothelial differentiation-related factor 1	EDF1	Breast, colorectal and prostate cancers

210986_s_at	tropomyosin 1 (alpha)	TPM1	Breast, colorectal and prostate cancers
212426_s_at	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide	YWHAQ	Breast, colorectal and prostate cancers
214214_s_at	complement component 1, q subcomponent binding protein	C1QBP	Breast, colorectal and prostate cancers
214224_s_at	protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting, 4 (parvulin)	PIN4	Breast, colorectal and prostate cancers
215111_s_at	transforming growth factor beta-stimulated protein TSC-22	TSC22	Breast, colorectal and prostate cancers
217848_s_at	pyrophosphatase (inorganic)	PP	Breast, colorectal and prostate cancers
217911_s_at	BCL2-associated athanogene 3	BAG3	Breast, colorectal and prostate cancers
218007_s_at	ribosomal protein S27-like	RPS27L	Breast, colorectal and prostate cancers
218286_s_at	ring finger protein 7	RNF7	Breast, colorectal and prostate cancers
33322_i_at	stratifin	SFN	Breast, colorectal and prostate cancers

EXAMPLE 5

Immunophenotyping of Leukocyte Carryover

In order to characterize leukocyte subsets carried over during immunomagnetic enrichment, EpCAM immunomagnetic selection was followed by further immunomagnetic selection using subset specific antigens to obtain proportional comparisons of leukocyte subsets. Amplification of the selected transcripts from EpCAM selected cells showed substantial signal interference from leukocyte contamination with epithelial cells.

Immunomagnetic selection of leukocytes subsets was used to obtain RNA from subset populations using CellSearch® Cancer Assay (Immunicon Corporation, Huntingdon Valley, Pennsylvania). Magnetic beads coated with antibodies directed against epithelial cells were used to isolate circulating epithelial cells from blood samples in the presence of an appropriate magnetic field. RNA was liberated from theses cells for amplification by RT-PCT (Table

4). This method is useful for obtaining satisfactory signal-to-noise in assessing genes found only in epithelial cells and not in leukocytes.

Table 4: Relative WBC background expression level in selected gene transcripts.

Gene	GenBank	Size (bp)	P1-nt to 3'	WBC 0	WBC 1	WBC 2	WBC 3	WBC 4
α-1-globin	V00491	451	580					100
AR	NM_000044	207	513	100				
CEA	M29540	144	297	100				
CK5	NM_000424	212	353	100				
CK19	NM_–2276	228	320	100				
EGFR	NM_005228	200	265	100				
ER-b	NM_001437	232	323	100				
HK2	XM_008996	190	334	100				
MGB1	XM_006409	340	408	100				
MGB2	NM_002407	264	420	100				
PSA	M26663	236	236	100				
PSGR	AF311603	281	425	100				
PSM	M99487	286	459	100				
TROP	X77753	245	334	100				
NKX3A	NM_006167	246	284	92	8			
MMP2	NM_004530	216	332	92	8			
Muc1	J05582	238	361	77	23			
Epcam	M33011	275	432	61	39			
Topo2a	NM_001067	244	356	54	46			
Mic1	AF019770	289	352	46	54			
MDR1	AF016535	215	263	23	62	15		
Hepsin	NM_002151	101	383	92			8	
TERT	NM_003219	234	347	92			8	

uPA	NM_002658	285	387	84		8	8	
ER-a	NM_000125	328	382	84	8		8	
PIP	J03460	201	226	76		8	8	8
Her2Neu	M11730	266	426	61	23	8	8	
CK8	M34225	275	429	46	31	8	15	
MMP9	NM_004994	215	327	30	39	8	15	8
MRP	L05628	300	328	23	23	31	23	
CK18	NM_000224	275	331	15	39	8	39	
TS	AB062290	114	398	15	15	31	39	
Timp2	NM_003255	219	325	8	31	46	15	
BCL2	XM_008738	330	440		23	46	31	
Topo2b	NM_001068	279	366		15	23	62	
Timp1	NM_003254	240	302		8	61	31	
p53	AF307851	243	334		8	8	30	54
CK10	NM_000421	196	305				100	

As shown in Table 4, a fraction of leukocytes are selected in addition to magnetically isolating epithelial cells. For every 7.5 ml of blood, 2,000 to 5,000 leukocytes are also selected with EpCAM immunomagnetic selection (about 0.005% to 0.01% of the leukocyte population). This small percentage contributes to background RNA interference after amplification of the total recovered pool. Information as to whether these specific genes are limited to leukocyte subsets or are universally retained throughout the leukocyte population would provide further insight into any analysis of their expression.

After EpCAM immunomagnetic selection, leukocyte subsets were selected by populations specific for CD3, CD4, CD8, CD14, CD15, CD20, and CD56. Resultant cell counts were determined, and the purity of selected population assessed using FACSCalibur flow cytometer. The collected cells were resuspended in 2.5 ml PBS for RNA analysis.

The results show that all major leukocyte subsets are present after EpCAM immunomagnetic selection. The proportions of leukocyte subsets, present in the carry-over, shifts from the expected proportion in average human blood (i.e. lymphocytes/monocytes to granulocytes is 40% to 60%, respectively) to an increase in lymphocytes/monocytes, possibly due to an increase in B-cells and monocytes (i.e. lymphocytes/monocytes to granulocytes is now 60% to 40%, respectively). This shift is present after EpCAM immunoselection in both normal donor blood samples and prostate blood samples.

Amplification of genes overexpressed in epithelial cells, yet still expressed in certain leukocytes may be relevant in disease diagnosis and treatment. The background noise from the leukocyte component contributes substantial interference to the amplification of these genes as they are expressed on isolated epithelial cells. The relative expression in leukocyte subsets and the carryover of these subsets are considerations in any genetic interpretation of circulating epithelial cells, especially after WBC subtraction.

These examples are several of many possible gene sets obtained through the embodiment of the present invention which can be exclusively expressed in specific cancer types like these (breast, prostate, or colorectal cancer), and potentially serve as cancer-specific CTC markers. Genetic information describing two or more cancer types may also serve as cancer-specific markers, but may further provide insight into a common thread between surveyed cancer types in the research and development of anti-cancer agents.

Accordingly, it is to be appreciated that the foregoing preferred embodiments of the present invention are not intended to be limitative of its scope, and that one skilled in the art will be able to conceive of various variations and modifications of such particular embodiments, all of which should be considered to be within the scope of the invention, which is limited solely by the following claims.

What is claimed is:

1. A method for detecting genetic information of rare cells in a biological sample comprising:

g. obtaining a biological sample containing a mixed population of cells from an individual suspected of having target rare cells;

h. fractionating said biological sample to obtain a fraction suspected of containing said rare cells;

i. assessing said fraction for a first gene profile;

j. separating said rare cells from said fraction whereby a depleted fraction is devoid of said rare cells;

k. determining a second gene profile of said depleted fraction; and

l. subtracting said second gene profile from said first gene profile to obtain said genetic information from said rare cells.

2. The method of claim 1 whereby said rare cells are from a group consisting of cancer cells, epithelial cells, endothelial cells, activated T-lymphocyte cells, dendritic cells and combinations thereof.

3. The method of claim 1 whereby said fraction is a white blood cell region from a density-partitioned blood sample.

4. The method of claim 1 whereby said separating is an immunomagnetic enrichment of said rare cell populations from said fraction.

5. The method of claim 1 whereby said assessing is by detection of hybridized genetic material in said fraction with an array of known genetic markers on a first fixed support.

6. The method of claim 1 whereby said determining is by detection of hybridized genetic material in said depleted fraction with said array of known genetic markers on a second fixed support.

7. The method of claim 1 whereby said subtracting is a direct comparative analysis of individual genes within said gene profile.

8. The method of claim 1 whereby said genetic information is indicative of cancer, cardiovascular disease, autoimmune diseases and combinations thereof.

9. A system for detecting genetic information of rare cells in a biological sample comprising:

a. means for obtaining a biological sample containing a mixed population of cells from an individual suspected of having rare cells;

b. means for fractionating said biological sample to obtain a fraction suspected of containing said rare cells;

c. means for assessing said fraction for a first gene profile;

d. means for separating said rare cells from said fraction whereby a depleted fraction is devoid of said rare cells;

e. means for determining a second gene profile of said depleted fraction; and

f. means for subtracting said second gene profile from said first gene profile to obtain said genetic information from said rare cells.

10. The system of claim 9 whereby said rare cells are from a group consisting of cancer cells, epithelial cells, endothelial cells, activated T-lymphocyte cells, dendritic cells and combinations thereof.

11. The system of claim 9 whereby said fractionating means is centrifugation which forms a density-partitioned blood sample.

12. The system of claim 9 whereby said assessing means is a first microarry chip.

13. The system of claim 9 whereby said separating means is an immunomagnetic particle antigenically linked to said rare cell.

14. The system of claim 9 whereby said determining means is a second microarry chip.

15. The system of claim 9 whereby said subtracting means by a comparision between fluorescent hybridization intensity signals of individual genes on said first microarray chip and said second microarray chip by the group consisting of manual inspection, automated fluorescent analysis, and combinations thereof.

16. The system of claim 9 whereby said genetic information is a diagnostic tool in assessing cancer, cardiovascular disease, autoimmune diseases and combinations thereof.

Figure 1

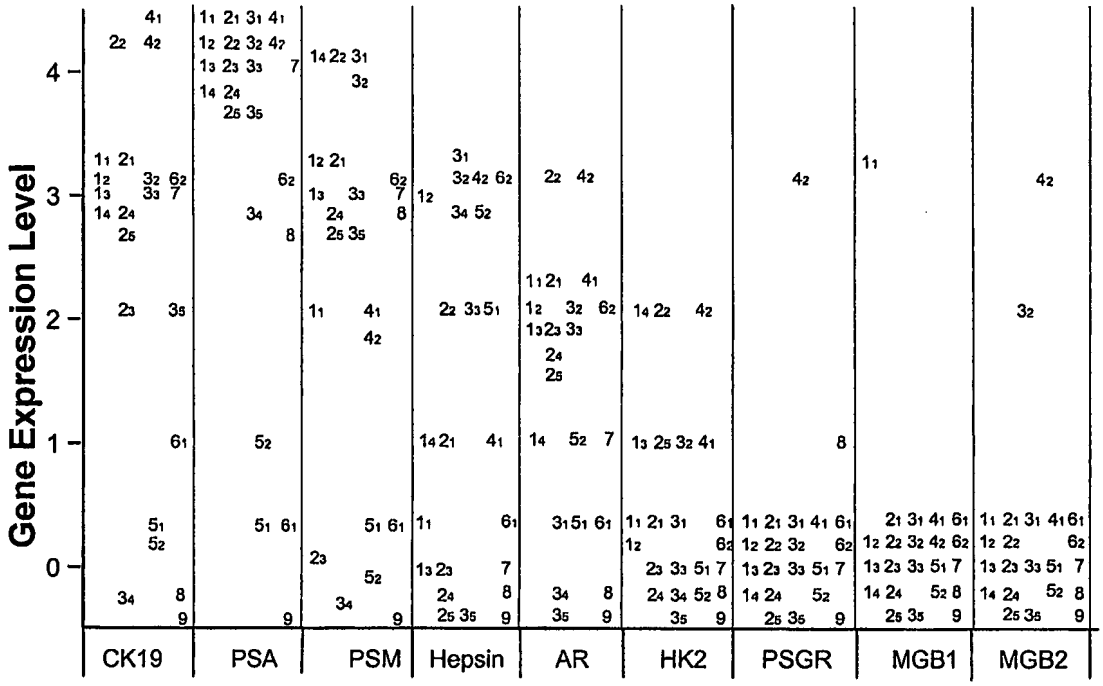
Gene Expression Level
4
3
2
1
0
CK19
PSA
PSM
Hepsin
AR
HK2
PSGR
MGB1
MGB2

Figure 2

Multigene RT-PCR sequence information and expression levels in SMART-aRNA libraries from immunomagnetically-enriched CTC from healthy donor control samples.

Marker Gene	GenBank accession	PCR Primer Pairs * Forward primer P1 / Reverse primer P2 Written 5' -> 3'	PCR fragment size-bp	aRNA ** required length-nt	Background *** Expression Level 0	1	2	3	4
a-1-globin	V00491	5-CTACTTCCCGCACTT / 10-TCAGCACGGT	451	580					100
AR	NM_000044	5-TGTGCAAGTGCCCAAGAT / 15-GACAGACTGT	207	513	100				
CEA	M29540	5-AAGATCAAGCAGACAAAA / 15-AAGAGTGGATAGC	144	297	100				
CK5	NM_000424	5-CTGCCTTCCAAGTGCAGCAA / 15-GATTTGAAGCAG	212	353	100				
CK19	NM_002276	5-CCAGGCGCTGATCAGCG / 10-CAGAAGCCCCAG	228	320	100				
EGFR	NM_005228	5-AACCTGACTGGTTAACAGCAG / 15-GGGAGTCA	200	265	100				
ER-b	NM_001437	5-CTGGCTCACTTGCTGAA / 10-GGCATTCAGCAT	232	323	100				
HK2	XM_008996	5-CATGCAGGATGACAT / 10-AGGTTCTCAG	190	334	100				
MGB1	XM_006409	5-AGCACTGCTACGCAGGC / 10-AGAGAAGGTGTGGT	340	408	100				
MGB2	NM_002407	10-ACTGCTATGCAGAT / 15-GTACACTGTATGCA	264	420	100				
PSA	M26663	5-CACTGAGCAGAAGCTGGA / 10-TGGAGGACTTCAA	236	236	100				
PSGR	AF311306	5-GCTTTGGAAACAGCCTTCATC / 15-TGGGCAACTGG	281	425	100				
PSM	M99487	5-AGTGAGAGACTCCAGGAC / 10-AAGGCTGCAACAT	286	459	100				
TROP2	X77753	5-CTACTCTGGTGTGTCCCAAG / 15-GGTACAGCTC	245	334	100				
NKX3A	NM_006167	5-TTCAGCCATCAGAAGTAC / 10-GTAAGGATAG	246	284	92	8			
MMP2	NM_004530	5-TGGCTGCCTTAGAACCTT / 10-TCGGTAGGGACAT	216	332	92	8			
Muc1	J05582	5-TGGCAGCAGCCTCTCTTA / 10-ACTGAGAAGTGTCCG	238	361	77	23			
Epcam	M33011	5-GCATAGGGAACTCAATGC / 10-CCAAGTTCTGGAT	275	432	61	39			
Topo2a	NM_001067	5-CCACTTCTGATGATTCTG / 10-GGCTTGGTAAGA	244	356	54	46			
Mic1	AF019770	5-ATCCCATGGTGCTCA / 10-ATCAGACCAG	289	352	46	54			
MDR1	AF016535	5-CCAGGCTGGAACAAAG / 10-TGATGTCTCTCAC	215	263	23	62	15		
Hepsin	NM_002151	5-AGGCGTCTACACCAA / 10-GGGTCACCAT	101	383	92			8	
TERT	NM_003219	5-ACCTGCCGTCTTCACTT / 10-TGGTCACTCCAA	234	347	92			8	
uPA	NM_002658	5-TGTGAGTGTAAGTGTGAG / 10-GGATTGGATGAAC	285	387	84		8	8	
ER-a	NM_000125	5-GTGCCTGAGACACAGA / 10-CGCTGGATTCTT	328	382	84	8		8	
PIP	J03460	10-CAAATTGCAGCCGTC / 15-TTCCAGCCAAG	201	226	76		8	8	8
Her2Neu	M11730	5-GGAAGAGGAACAGCACTG / 10-CTGACACCATTGC	266	426	61	23	8	8	
CK8	M34225	5-TTGAGCTCGGCCTATGG / 10-CCTGCATAGCG	275	429	46	31	8	15	
MMP9	NM_004994	5-TCCAGTACCGAGAGAAAG / 10-AAACTGGCTCCTT	215	327	30	39	8	15	8
MRP	L05628	5-TCGTCTTGGACAAAGGAG / 10-CAGTTCCAGGCAG	300	328	23	23	31	23	
CK18	NM_000224	5-GAGTCAGAGCTGGCACAGA / 10-GCTTAATGCCTCAG	275	331	15	39	8	39	
TS	AB062290	5-CTGGCAAATGTAACTGT / 10-TCCTCACTTTGTTCAT	114	398	15	15	31	39	
Timp2	NM_003255	5-TGCGAGTGCAAGATCAC / 10-GTCCTCGATGTC	219	325	8	31	46	15	
BCL2	XM_008738	5-AGTGACAGTGGATTGCAT / 15-TGGAGACT	330	440		23	46	31	
Topo2b	NM_001068	5-CAAGAGAGCCCCAAAAC / 10-GGTGGCTCAGTA	279	366		15	23	62	
Timp1	NM_003254	5-ACCTACACTGTTGGCTGT / 10-CTTCAGTTCCACT	240	302		8	61	31	
p53	AF307851	5-TCAGCCTCCGGAGTAGCT / 10-AATGCAGATGTGC	243	334		8	8	30	54
CK10	NM_000421	10-TTCTTTCATCTACGGTTG / 15-CCTTGTAGACACC	196	305				100	

* PCR primer pair column shows the truncated versions where the 5' numbers (5,10,15) indicate the number of deleted nucleotides, using GenBank accession number, primer sequence and PCR size the complete sequences can be determined.

** Column indicates the minimum aRNA fragment length in nucleotides (nt) required for each gene to be amplified successfully by RT-PCR using the corresponding PCR primer pair.

*** Indicates the expression level for the genes in CTC enriched blood samples from 13 healthy individuals which composition consists of leukocytes carried over during the procedure. Expression is indicated as a percentage of the signals relative to the external standard curve.